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## **Heparanase in Cancer Metastasis - Heparin as a Potential Inhibitor of Cell Adhesion Molecules**

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**Abstract:** Tumor progression associated with hematogenous metastatic spread is a multistep process based on a cross-talk between tumor and stromal cells in a tumor microenvironment. In the blood circulation, tumor cells interact with blood cells through receptors such as selectin and integrins that promote tumor cells survival. At the metastatic sites, heparanase secreted by tumor or stromal cells is an important modifier of the tumor microenvironment while promoting tumor invasiveness and angiogenesis. Heparin, particularly low molecular weight heparin, is used for treatment of cancer patients with evidence of hypercoagulability. However, in preclinical studies heparins was shown to contain other biological activities that affect cancer progression including inhibition of heparanase, selectins and integrins. While ongoing clinical trials are assessing inhibition of heparanase on cancer progression, the remaining biological activities of heparins inhibiting cells adhesion, through selectins and integrins remains largely unexplored. This chapter addresses the potential role of heparins in oncology with respect to their anti-heparanase and anti-adhesive activities and aims to discuss aspects relevant for broader therapeutic application of heparins.

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**Book chapter**

**Heparanase in cancer metastasis –heparin as a potential inhibitor of cell adhesion molecules**

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## **Introduction**

Cancer progression – metastasis is a process encompassing multiple steps including successful escape of tumor cells from the primary tumor sites, survival in the circulation, evading immune responses, seeding in distant organs, and most importantly initiation and a sustained growth at these sites. Even though sustained proliferation of tumor cells is likely the most fundamental trait in tumor cells, the capacity to “modulate” the tumor microenvironment consisting of non-tumorigenic stromal cells significantly contributes to metastasis, virtually at every step of this process (1, 2). Particularly, the capacity of tumor cells to secrete extracellular matrix degrading enzymes, such as heparanase or proteases, profoundly contribute to migratory properties of tumor cells, or to the release of factors promoting tumor growth and angiogenesis (3, 4).

Heparanase expression is linked to an invasive phenotype of a variety of cancer types in patients and has been confirmed in numerous animal models (3, 5, 6). This book covers all known aspects of heparanase biology in great details, thus the focus of this chapter is on heparanase action in leukocyte recruitment and cell-cell interactions during cancer progression. In addition, the activity of heparin and heparin derivatives on cancer progression will be discussed with respect to their anti-heparanase and anti-adhesive activities; and current developments towards therapeutic applications.

## **Cell adhesion promotes tumor cell and leukocyte migration**

The immune system "responds" only when leukocytes are able to cross blood vessels. Circulating leukocytes do not interact with the endothelial cells. Endothelial activation is required to initiate leukocyte adhesion and to enable the migration of leukocytes through the endothelium. The formation of chemokine gradient is a prerequisite for driving the cell recruitment and the capacity of cells to adhere to the vessel wall and to initiate trans-endothelial migration (7). Selectins are likely the first cell adhesion molecules

involved in leukocytes recruitment and transendothelial migration. Inflammatory stimuli activate endothelial cells that increase expression of adhesion molecules including P-selectin, E-selectin and increases vascular permeability (8, 9). Interactions of vascular selectins with their ligands lead to the leukocyte rolling on activated endothelium, followed by integrin-mediated firm adhesion and finally leukocyte extravasation into the parenchyma, where leukocytes can execute their effector functions (10).

Extravasating leukocytes need to engage the endothelium, and after diapedesis they face the basement membrane surrounding most of postcapillary venules, which is a complex meshwork of collagens interconnected with glycosaminoglycans, such as heparan sulfate. Heparanase is a hydrolytic enzyme enabling degradation of heparin sulfate, thus likely promoting leukocyte migration after extravasation (11, 12). Interestingly, neither neutrophils nor T cells require heparanase expression for an efficient extravasation as has been shown using heparanase deficient mice (11). On contrary, efficient monocyte extravasation required heparanase expression, in a peritoneal inflammation model. In the pulmonary vasculature, which represents the largest capillary bed in our body, the transendothelial migration of neutrophils also does not require heparanase expression (12). However, in chronically inflamed lungs, neutrophil accumulation required heparanase, while T cell recruitment remained heparanase independent but was mediated by ICAMs instead. Thus, on contrary to tumor cells, where enhanced heparanase expression is linked to an invasive behavior, leukocyte transendothelial migration does not require heparanase.

During cancer metastasis selectin-mediated interactions were shown to be essential for the recruitment of myeloid cells and monocytes that enhances tumor cell extravasation and facilitate metastasis in several mouse models. The metastatic microenvironment further promotes recruitment of monocytes and myeloid cells through enhanced presence of chemokines, e.g. CCL2, CCL5 (9, 13). These observations show that metastatic tumor cells “highjack” the physiological function of selectins and leukocyte recruitment to promote tumor growth and metastasis.

## **Cell adhesion as determinant of metastasis**

Cell adhesion defines the physiological function of any cell in the body through contacts to other cells or to extracellular matrix (ECM) component within the tissue environment. Since cell adhesion is connected to the signal-transduction pathways affecting cell phenotype, survival, differentiation and migration, alteration in cell adhesion frequently observed in tumor cells directly contributes to cancer progression. During cancer progression several families of adhesion molecules including: cadherins, integrins, junctional-adhesion molecules, and selectins have been studied. The topic of cell adhesion in cancer progression is too extensive to be covered in this chapter and excellently reviewed elsewhere (14, 15). Thus, we focus on the role of cell adhesion in steps of metastasis in context of potential involvement of heparanase.

Hematogenous metastasis is a highly orchestrated process describing the ability of tumor cells to enter in the blood circulation, survive the circulation and extravasate in secondary sites, where they can form metastases. Tumor cells in circulation undergo multiple interactions with blood cellular components, such as platelets and leukocytes that are essential for tumor cell survival and further for metastasis. Two major families of cell adhesion molecules, selectins and integrins, have been identified to facilitate cell-cell interactions essential for the metastatic spread (reviewed in (10, 16-19). Here we will discuss selectins and integrins both as mediators of metastasis and as targets of heparin-based therapeutic approaches.

### *Selectin as mediators of metastasis*

Selectins have been identified as key adhesion molecules that mediate adhesive events between leukocytes, endothelial cells and platelets during leukocyte trafficking and hemostasis (20). There are three members of the selectin family: P-, L- and E-selectin. Platelets and endothelial cells express P-selectin that is stored in  $\alpha$ -granules and Weibel-Palade bodies, respectively; and upon activation rapidly translocates

on the cell surface of these cells. E-selectin is expressed in endothelial cells, where upon activation a *de novo* transcription is initiated, and its cell surface expression lasts longer than of P-selectin. L-selectin is constitutively expressed on cell surfaces of almost all leukocyte subpopulations (21). Selectins mediate adhesion by heterotypic interactions of their C-type lectin domain with glycan-bearing ligands. The minimal recognition motif for all selectins is the sialyl-Lewis<sup>x</sup> sLe<sup>x</sup> and its isomer sialyl-Lewis<sup>a</sup> (sLe<sup>a</sup>) tetrasaccharide that is sequentially synthesized by N-acetyl-glucosaminyltransferases, galactosyltransferases,  $\alpha$ 1,3-fucosyltransferases IV or VII, and  $\alpha$ 2,3-sialyltransferases (10, 21). Due to the relatively low binding affinity towards a single carbohydrate domain, physiological ligands represent a scaffold of clustered domains to increase the avidity of binding. In addition, P- and L-selectin, but not E-selectin, can bind to sulfated glycans including heparin, heparan sulfate, fucoidan and sulfated glycolipids (22), indicating that selectins recognize rather a carbohydrate “patch” generated in different ways.

The relevance of selectin involvement in hematogenous metastasis has been deduced from the observations that particularly epithelial cancers, carcinomas undergo profound changes in cell-surface glycosylation (23, 24). The cancer-induced aberrant glycosylation often goes along with enhanced presence of selectin ligands enabling tumor cells to interact with other blood constituents once they enter the blood circulation (23). Selectin-mediated interactions of tumor cells with leukocytes, platelets and endothelial cells provide a mechanistic explanation for the clinical association with poor prognosis for cancer patients (23, 25, 26). Tumor cells in blood circulation are often covered by platelets, as has been observed in patients and animal models. The first evidence that this platelet-tumor cell interaction is P-selectin dependent has been described in a mouse model with P-selectin deficiency (27). Platelet's P-selectin bound to carcinoma mucins, since glycan removal inhibited platelet-tumor cell interaction and thus also metastasis (28). The role of platelets in malignancy and metastasis has been thoroughly investigated since then and is excellently reviewed elsewhere (29-31). Of note, P-selectin expression on endothelial cells also contributes to metastasis as has been shown in bone marrow reconstituted P-

selectin-deficient mice (32). Similarly, E-selectin has been associated with formation of a premetastatic niche, where the recruitment of tumor cells as well as myeloid-derived cells facilitates metastasis (33, 34). Interestingly, the endothelial activation is essential for the recruitment of monocytes and tumor cell extravasation (9, 35).

Leukocytes are a key component of the tumor microenvironment and they exert many activities promoting metastatic dissemination and metastatic niche formation. Particularly, myeloid-derived cells such as monocytes and neutrophils expedite tumor cell extravasation and formation of metastatic niche (36-38). L-selectin facilitates the recruitment of myeloid cells to tumor cells in circulation and enables their extravasation through the endothelial cells (13, 39). The inhibition or the absence of L-selectin resulted in attenuation of metastasis due to the lack of leukocyte-induced endothelial activation (9, 13). There is little knowledge about the role of L-selectin on lymphocytes in cancer progression, despite the canonical function of L-selectin in facilitating of lymphocytes recruitment into lymph nodes (21). In an inflammation model, L-selectin was shown to mediate the recruitment of activated CD8<sup>+</sup> T cells to virus-infected organs and thereby confers protective immunity (40). Whether L-selectin facilitates activated cytotoxic CD8<sup>+</sup> T cell recruitment during tumor progression remains unclear.

#### *Selected aspects of integrins during cancer metastasis*

Integrins are ubiquitously expressed trans-membrane glycoproteins with important functions in cellular adhesion and signaling. The structure of integrins comprises a non-covalently bound heterodimers with  $\alpha$ - and a  $\beta$ -subunit. 18  $\alpha$ - and 8  $\beta$ -integrin subunits have been characterized that combine to form 24 unique canonical  $\alpha/\beta$  receptors identified so far. Integrins mediate cell adhesion, primary to components of the ECM, such as fibronectin, vitronectin, laminin, or collagen, thus contributing to cellular anchorage but also to cell motility and invasion. Furthermore, integrins also mediate certain aspects of cell-cell

interactions relevant for tumor cell metastasis. Integrins are important mediators of a bidirectional cellular signaling due to their anchorage to the cytoskeletal structures (e.g.  $\alpha$ -actinin, talin, and vinculin). As a consequence, ligation of extracellular ligands can influence intracellular processes (outside-in signaling) through activation of kinases, GTPases of the Ras/Rho signaling pathways. On contrary, intracellular signals can induce alterations in the integrin conformation and thus change ligand-binding properties (inside-out signaling).

An immense body of knowledge has been accumulated covering the multiple roles of integrins in oncology and tumor cell metastasis recently reviewed (41). To name just few aspects of integrin-triggered interaction during metastasis, integrins mediate: growth factor receptor signaling (42), tumor cell chemoresistance (43), epithelial to mesenchymal transition (44) and angiogenesis (45). For further details we refer the readers to excellent reviews in this field (41, 46). In general, the expression pattern of integrins has been associated with the malignant progression of certain tumors and correlated with altered patient's survival. In this context, we will address here only the functions on integrins, which are directly related to cell-cell interaction during metastasis and discuss the potential targets for heparin to interfere in these processes.

The role of platelet in cancer metastasis has been outlined regarding of P-selectin. However, platelets also express five different integrins, which contribute to the formation of tumor cell-platelet emboli during metastasis (47). For instance,  $\alpha 6 \beta 1$  and  $\alpha IIb \beta 3$  integrins are directly involved in platelet adhesion to tumor cells and pharmacological interference with these integrins resulted in attenuated metastasis (48, 49). The  $\alpha 4 \beta 1$  integrin, very-late antigen-4 (VLA-4), is found on many cells of hematopoietic origin referring to their role in immune response by binding to the endothelial ligand VCAM-1. In addition, VLA-4 is also aberrantly expressed and active in different tumor types, such as melanoma (50). The current evidence show that VLA-4 facilitates melanoma cell binding to activated endothelial cells expressing VCAM-1 at distant sites; and the interference in VLA-4 attenuates melanoma metastasis in different model



systems (51-54). Furthermore, the aberrant expression of the VLA-4 ligand, VCAM-1, by certain tumor cells was shown to mediate trafficking and binding of macrophages into the forming micrometastases and thereby promotes the formation of a permissive microenvironment for tumor cell outgrowth (55). Integrins have recently been identified to contribute to organ-specific metastasis through their expression on tumor-derived exosomes (56). Proteomic analysis of tumor-derived exosomes with tropism towards lungs and liver were shown to express predominantly the integrins  $\alpha 6\beta 4$  and  $\alpha 6\beta 1$  (lungs) and  $\alpha v\beta 5$  (liver), driving the formation of a pre-metastatic niche in the respective organ.

Taken together, selectin and integrin adhesion receptors promote cancer progression through various mechanisms (reviewed in (16)). As a consequence, a pharmacological interference with adhesion receptor activities remains an attractive option to attenuate metastasis. Although the inhibition of selectins in experimental settings has been efficient in numerous cancer models, there are no clinical studies focusing on selectins. In case of integrins, the ubiquitous expression of the different integrin subtypes and partly overlapping ligand recognition complicate a specific targeting. Interestingly, heparin and heparin derivatives have been shown to inhibit P- and L-selectin as well as VLA-4 integrin in experimental settings. Since heparins are currently used in treatment of cancer patients with thrombosis, the question remain to which extent heparin may affect cell adhesion or heparanase activity during metastasis.

### **Heparin as inhibitor of cell adhesion**

Heparin, low-molecular weight heparin (LMWH) and heparin derivatives (further named as heparins) were tested in many different animal models for its potential to attenuate cancer progression (reviewed in e.g. (57, 58)). Interestingly, various heparins tested in animal models have shown primarily attenuating effect on metastasis rather than tumor growth. Most of these studies were performed in an experimental metastasis model where tumor cells were directly injected into the blood circulation. Despite

many limitations of this experimental approach, the timely defined presence of tumor cells in the circulation has allowed the evaluation of cellular and molecular mechanisms during the hematogenous phase of the metastasis. A variety of heparins efficiently reduced cancer progression when applied shortly before or shortly after the intravenous injection of tumor cells (57). However, the application of heparin twenty four hours before or after the tumor cell injection had no effect on metastasis (32, 59). While in the majority of early studies heparins were used at concentrations exceeding the therapeutic dosage range, later publications have confirmed the efficacy of heparin to inhibit metastasis also at clinically relevant concentrations (60, 61). The fact that heparins showed anti-metastatic activity only when tumor cells were still in circulation strongly indicate that cellular and molecular events occurring during this phase are potential targets of heparin instead of solely affecting the coagulation pathway (16, 26). This conclusion is further supported by the observation that half-life of heparin in circulation is rather limited, not exceeding six hours, thus affecting processes like angiogenesis or tumor growth less likely.

Heparin is a complex natural glycosaminoglycan extracted from porcine intestine, which in clinical preparation is enriched for the ability to inhibit the clotting cascade. In addition, heparins have a wide variety of potential biological effects including the ability to: interact with integrins, inhibit P- and L-selectin interactions, inhibit heparanase, attenuate angiogenesis; and affect growth factors and chemokines (26, 62). Despite these many potential effects, experimental data indicate that heparins affect processes during the hematogenous phase of metastasis. Within blood vessels, circulating tumor cells ultimately interact with the endothelium and other blood constituents (e.g. platelets and leukocytes) that might lead to tumor cell arrest and extravasation also through increased heparanase activity. Thus, the antimetastatic activities of heparins were analyzed by various groups for their potential to affect coagulation, inhibit cell-cell interactions and to block heparanase [as depicted in Figure 1](#) (reviewed in 63). Although the anticoagulant activity may contribute to reduced metastasis, several works clearly indicated that heparins without anticoagulant activity attenuated equally well cancer progression in various cancer entities; e.g. lung, colon

and breast cancers (reviewed in 64). The initial finding that P- and L-selectins can effectively bind to heparins (65, 66) initiated a series of studies testing the hypothesis that heparin treatment inhibits selectin-mediated interactions and thereby metastasis (28, 39, 60). In parallel, it was shown that integrin-mediated interactions during inflammation can be inhibited by heparin (67, 68), suggesting that heparin may block also integrin-mediated interactions during metastasis. Indeed, heparin was shown to block  $\alpha\text{IIb}\beta\text{3}$ -integrin mediated interactions of platelets with melanoma cells (69). In another study, heparin was shown to block  $\alpha\text{4}\beta\text{1}$ -integrin mediated adhesion of melanoma cells to the endothelium (70). Taken together, these studies provided a rationale to explore heparin as an inhibitor of cell adhesion during metastasis, albeit the heparanase-inhibitory activity remained to be answered.

### **The role of heparin in cancer treatment - Clinical evidence**

The close relationship between cancer and hypercoagulability has been observed already in the 19<sup>th</sup> century by the French physician Armand Trousseau (reviewed in (71, 72) and is under constant investigation since then (reviewed in (73, 74). Cancer patients with malignant diseases are at a higher risk of developing thromboembolic complications contributing to morbidity and mortality of the disease when compared to healthy population. This includes venous thromboembolism (VTE) which encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE); and also arterial thrombosis. Cancer-associated thrombosis in numbers is reflected in six time higher chance to develop VTE compared to non-cancer subjects, that is further increased in patients receiving chemotherapy (75). Epidemiologically, about 15% of cancer patients will develop VTE, and about 20% of patients with VTE have an unknown neoplasm at the time of diagnosis. Consequently, VTE complications contribute significantly to morbidity and mortality of cancer patients, where VTE is the second leading cause of death. There are several mechanisms being identified to be involved in cancer-triggered thromboembolism enabling development of specific therapies (reviewed in 72, 75).

Antithrombotic prophylaxis or treatment is an essential component of clinical therapy especially for patient populations with an increased risk to develop VTE. A multitude of studies and meta-analyses have been performed to define an optimal pharmacological interference with the activated hemostatic system in cancer diseases (reviewed in (63)). The treatment guidelines of first-line therapy for the short- and long-term management of cancer associated VTE, including these of the European Society of Medical Oncology (ESMO), the National Comprehensive Cancer Network (NCCN), or the American Society of Clinical Oncology (ASCO) currently recommend LMWH as first choice (76, 77). The clinical handling of LMWH in oncology is experienced for several decades and displays a balanced safety profile, e.g. superiority over vitamin K antagonists. The guidelines recommend the treatment of acute symptomatic VTE by LMWH for longer time periods, up to six months. In terms of VTE prophylaxis, LMWH is also guideline-based recommended for certain cancer patients for short term application (78). Although heparin and particularly LMWH are used for treatment of cancer patients with thrombotic complication for decades, the question, whether heparin treatment has other anti-cancer activities, going beyond anticoagulation, remains open.

A potential antitumor effect of an antithrombotic treatment using heparin and LMWH has been suggested based on several retrospective and prospective clinical trials of cancer patients at risk of VTE (summarized in 79). A number of controlled prospective studies in the early 2000 years showed promising results especially in patients with early, non-metastatic stage of the disease. While there was no effect on overall survival in variety of solid tumors, the analysis of a subset of patients, who were metastasis-free at the beginning of the trial, demonstrated a significant increase in survival with the LMWH dalteparin in that population (80-83). An open-label controlled study in small cell lung cancer patients, receiving standard treatment plus/minus LMWH in a therapeutic dose for 18 weeks, showed a significant increase in the overall survival and progression free survival in the treatment arm (84). A comprehensive review on all clinical trials conducted by 2007 has been re-evaluated that confirmed certain benefits for LMWH-treated

cancer patients especially in the early stage of lung cancer (85). Nonetheless, several further clinical studies could not, or not completely confirm this beneficial LMWH effect on overall survival, while the reduction in the VTE as a primary endpoint by LMWH was significant (86-88). Three of these studies have been performed in non-small cell lung cancer patients using three different LMWH preparations: nadroparin, dalteparin and tinzaparin, respectively (86, 87, 89).

The reasons for this non-favored outcome in light of LMWH are likely multifaceted due to methodological issues, tumor entities, or treatment regimens; and the use of different LMWH preparations. Furthermore, it remains open to which extent the chemotherapy, which was given in parallel, interferes with any effect of LMWH on patient's survival. Finally, it needs to be considered that all LMWH preparations are tested only for anticoagulant activity, but not for the other biological activities, such as inhibition of cell adhesion or heparanase. There is a considerable difference in effectiveness of different LMWH preparation on cell adhesion and subsequently on metastasis as tested in preclinical models (60). Nevertheless, further studies on LMWH are warranted to address the question, how heparins affect cancer progression, beyond coagulation. The design of a clinical study should take into account the biological activity of heparins that has been proven in numerous preclinical studies.

### **Heparanase – another player in cancer progression**

Heparanase (HPSE), an endoglycosidase, is the sole enzyme in mammalian organisms able to cleave heparan sulfate (HS) chains into HS fragments (90). HS macromolecules have an essential role in cell signaling and communication, primarily due to their capacity to bind to growth factors and cytokines and thereby creating a reservoir of signaling molecules in the ECM and on the surface of cells. Thus, the capacity of HPSE to degrade HS has important implications for remodeling of the cellular

microenvironment as has been shown during inflammation and cancer progression (91). HPSE affects tumor growth and metastasis in various ways, e.g. by fostering tumor cell extravasation, angiogenesis etc. (discussed in other chapters of this book). Many types of cancer show upregulated expression of HPSE that is a marker of poor prognosis in cancer patients (92). Experimental evidence using transgenic expression of HPSE both in tumor cells or in mice as well as HPSE knock-down methods convincingly linked HPSE to metastasis (93, 94). Interestingly most but not all activities of HPSE during tumorigenesis and metastasis were shown to be related to the enzymatic functionality altering the tumor microenvironment. Here we briefly recapitulate the most important aspects of HPSE with respect to metastasis, while a thorough discussion of HPSE protumorigenic activities can be found in other chapters of this book and are reviewed elsewhere (5, 95).

The enzymatic function of HPSE was shown to promote tumor angiogenesis and production of exosomes that significantly contribute to formation of metastasis (96, 97). Recently it was shown that HPSE-mediated shedding of syndecan-1 liberates peptide fragments with VEGF receptor activity (98). Exosomes are dominant mediators of intercellular communication that drive metastasis by regulating the tumor-host cell interactions both locally within the tumor microenvironment and distally at metastatic sites (99). The enhanced expression of HPSE in human cancer cells, as well as tumor cell exposure to exogenous HPSE possibly derived from the tumor microenvironment, was shown to dramatically increase exosome secretion through a modulation of syndecan-1 signaling (97, 100). This process relies on the HPSE enzymatic cleavage of heparan sulfate, and also impacts exosome protein cargo as reflected by higher levels of syndecan-1, VEGF and HGF (97). HPSE was identified as a key factor in myeloma cells driving survival and resistance against chemotherapy via activating the ERK signaling pathway (101). Treatment of colon carcinoma cells with heparanase induced expression of inflammatory cytokines such as CCL2, CCL5 and CXCL1, indicating that heparanase from stromal cells has the capacity to directly activate tumor

cells and thereby modulate the tumor microenvironment (102). Similarly, heparanase activity was shown to activate macrophages in a tumor microenvironment through the Erk, p38 signaling pathway (103).

With respect to non-enzymatic activity of HPSE, latent HPSE induces adhesion receptor activity for spreading or migration of different tumor cell types by inducing a signaling axis via binding and clustering the cellular HSPGs (reviewed in 3). Latent HPSE facilitates integrin binding and thereby promotes adhesion and metastasis of melanomas, which can be antagonized by LMWH (104). Interestingly, latent HPSE on endothelial and cancer cells induces tissue factor (TF) expression and thus contributes to coagulation (105). Furthermore, the latent HPSE binds and displaces tissue factor pathway inhibitor from the endothelial surface as yet another way to modulate thrombosis in cancer (106).

The multiple functional mechanisms how HPSE facilitates tumor progression and metastasis make it an excellent target for pharmacological inhibition. Manifold experimental approaches exist at the preclinical and even clinical level to affect HPSE in a therapeutic perspective. Not surprisingly, heparins have been one of the first targets tested in many *in vitro* and *in vivo* models.

### **Heparin as an inhibitor of heparanase in metastasis**

The role of ECM-bound or cell surface-bound proteoglycans in cancer progression has granted the development of HPSE inhibitors on the basis of heparin structure. Structural evaluations have led to identification of two high affinity heparin recognition domain of HPSE, one closed to the N-terminus of the enzyme, where blockade of binding to heparin inhibits the enzymatic activity (107). Meanwhile, in 2015 the crystal structure of HPSE has been resolved providing a further insight into the mapping of substrate recognition sites (108).

Heparin has served as a structural scaffold for optimization of the structural requirements for the development of glycosidic inhibitors of HPSE. First structural modifications of heparin identified the requirements for sulfation and N-acetylation to differentiate between anticoagulant and HPSE inhibitory properties of heparin during hemostasis (109, 110). Later on, the capacity of heparin and heparin derivatives to attenuate the lung colonization by melanoma cells has been confirmed using experimental metastasis approach in mice (59). The effectivity of heparins to attenuate metastasis was restricted to a short time period shortly before and several hours after tumor cell inoculation, which was considered to inhibit predominantly the tumor cell-derived HPSE during this phase. Another group has confirmed the impact of sulfation degree of heparin derivatives on HPSE inhibition in the same melanoma experimental metastasis model (111). Notably, in this study, the opening of the iduronic acid ring by oxidative/reductive procedures has been prepared and tested for its HPSE inhibitory activity. This approach has been later identified as one of the key methods to optimize heparin for its HPSE inhibitory activity (112).

A comprehensive library of heparin derivatives as potential HPSE inhibitors has been assessed *in vitro* (112). The essential structural requirements of heparin for HPSE inhibition has been linked to its sulfation grade. A partial N-desulfation (replaced by acetylation) is well tolerated and considered as a discriminating factor between anticoagulant and HPSE-inhibiting activity of heparin. However, the preparation of a series of glycol-splitting derivatives (RO-heparins) with various degrees of N-desulfation resulted in identification of highly efficient compounds (112). These derivatives were used as scaffolds for development of HPSE-specific inhibitors (outlined in the [chapter number 35 of this book](#)) such as roneparstat that has been tested in multiple myeloma patients (113). The elimination of conformational restrains by glycol-splitting of the iduronic acid leads to rotational freedom of the heparin molecule, which together with N-acetylation strongly enhanced HPSE binding and thereby inhibitory potential. [The structural modifications of heparin discussed with respect to all discussed biological activities are schematically shown in Figure 2.](#) A further insight into the role of structural flexibility of glycol-split



derivatives has recently been given by a NMR-based conformational analysis and molecular dynamic study (114).

Glycol-split heparin derivatives with various degree of N-desulfation/N-acetylation have been tested for their antimetastatic activity (115). RO-heparin as a specific inhibitor of HPSE inhibited melanoma metastasis, while had little effect on colon carcinoma metastasis. Interestingly, melanoma cells express high levels of HPSE while there was little expression detected in colon carcinoma cells, indicating the efficacy of HPSE inhibition only in cancers associated with high HPSE expression. Further studies investigated heparin-like polymers from mollusk origin for their capacity to inhibit HPSE besides other potential targets and thereby metastasis (116). Novel strategies developing HPSE inhibitors based on heparin-like structures resulted in preparation of a glycopolymer with specific sulfation pattern to be active in attenuating breast cancer metastasis (117).

Several preclinical studies using a variety of animal models confirmed the role of HPSE in metastasis (reviewed in 64, 118). Based on many studies developing heparin-based inhibitors of HPSE without anticoagulant activities resulted in development of two drugs: roneparstat and necuparanib, that are currently in clinical trials as a treatment of cancer with enhanced HPSE activities (113, 119). The relevance of HPSE inhibition and its impact on cancer progression is clearly defined. Nevertheless, heparin-based inhibitors require further analysis to determine the contribution of other biological activities (e.g. cell adhesion) to anti-metastatic activity.

### **Dissecting the role of heparin in cancer progression**

Heparin and its derivatives are being studied for their potential to treat cancer in numerous preclinical models, and the results of these studies clearly indicate the capacity of heparin to inhibit cancer progression independently of its anticoagulant activity. Since heparin, particularly LMWH, is still used for

treatment of cancer patients with thrombotic complications, and based on several studies is associated with prolonged survival of some patient groups, heparin remains an attractive option for further development. Many pre-clinical studies have shown that heparin derivatives, or non-anticoagulant heparin-based analogues attenuate metastasis irrespective of an animal model or cancer entity (59, 60, 115, 120-122). As discussed in this chapter inhibition of cell adhesion and of HPSE are likely the two biological activities contributing most significantly to inhibition of cancer progression. While several studies characterized heparin derivatives for their HPSE-inhibitory activity (e.g. 59, 120), other studies evaluated the selectin inhibitory activity (28, 60). Later on, heparin derivatives were tested for both HPSE and selectin-inhibitory activity using experimental metastatic mouse models (115, 122). The selectin-specific heparin derivative (57% N-acetylated-heparin) attenuated metastasis both with colon carcinoma and melanoma cells (115). On contrary, HPSE specific heparin derivative (100% N-acetylated glycol-split heparin) effectively inhibited melanoma metastasis, but was ineffective for attenuation of colon carcinoma metastasis. When we used semisynthetic sulfated trimannose C-C dimers, HPSE specific derivative again attenuated only melanoma metastasis, while selectin specific-derivative inhibited both melanoma and colon carcinoma metastasis (122). Although melanoma cells (B16-BL6) expressed significant amounts, colon carcinoma cells (MC-38) showed low amounts of HPSE (115). Of note, many tumor cells tested express high levels of HPSE (115, 123, 124). Taken together, these studies provided evidence that heparin-based selectin inhibition attenuated metastasis in both tested cells, while the inhibition of HPSE affected metastasis of those tumor cells with enhanced HPSE activity.

In a recent work, the HPSE-neutralizing antibody has been tested as inhibitors of cancer progression using Burkitt's lymphoma and glioma cells (123). While Raji cells do not produce any detectable HPSE activity, the anti-HPSE antibody significantly reduced tumor growth and metastasis of these cells. These findings indicate that heparanase derived from the tumor microenvironment is

significantly contributing to the tumor growth (123). Since primary tumor growth was significantly attenuated in this model, the specific role of heparanase inhibition on metastasis could not be addressed.

## **Conclusions**

Metastasis is in ninety percent of cancer patients the ultimate cause of death, yet there is no specific anti-metastatic therapy currently available. Based on the current understanding of metastasis, invasiveness and mechanisms related to tumor cell survival, HPSE; as the principal modifier of the tumor microenvironment and invasiveness; and cell adhesion mechanisms enabling tumor cell interactions with the other cells during transition, offers the best targets for exploration of future therapies. While specific targeting of HPSE, based both on heparin-glycomimetics and specific antibody-based therapies are ongoing, particularly in hematopoietic malignancies (5, 123, 125), there is little progress on cell adhesion-based approaches. However, heparin derivatives have been extensively studied for their capacity to interfere with cancer progression (reviewed in 10, 16, 26).

As discussed in this chapter, heparin carries several biological activities that are beneficial for attenuation of metastasis. In fact, most of heparin preparations used both in preclinical and clinical studies carried out thus far contained various activities such as HPSE and selectin-inhibitory activity. Yet, only the anti-coagulation and HPSE activity of heparin was mostly assessed. Despite of many studies, the identification of the critical biological activities of heparin for its anti-metastatic behavior is still not possible, largely due to the fact that heparin has not been vigorously tested for all or at least several known activities. Clearly, it is of scientific interest to dissect the role of heparin as an inhibitor of cancer progression, but from the clinical perspective, inhibition of multiple mechanisms involved in cancer progression by heparin might prove beneficial for the cancer patient. Thus, further clinical studies designed

according to the current knowledge of the potential mode of action of heparins in cancer setting are warranted.

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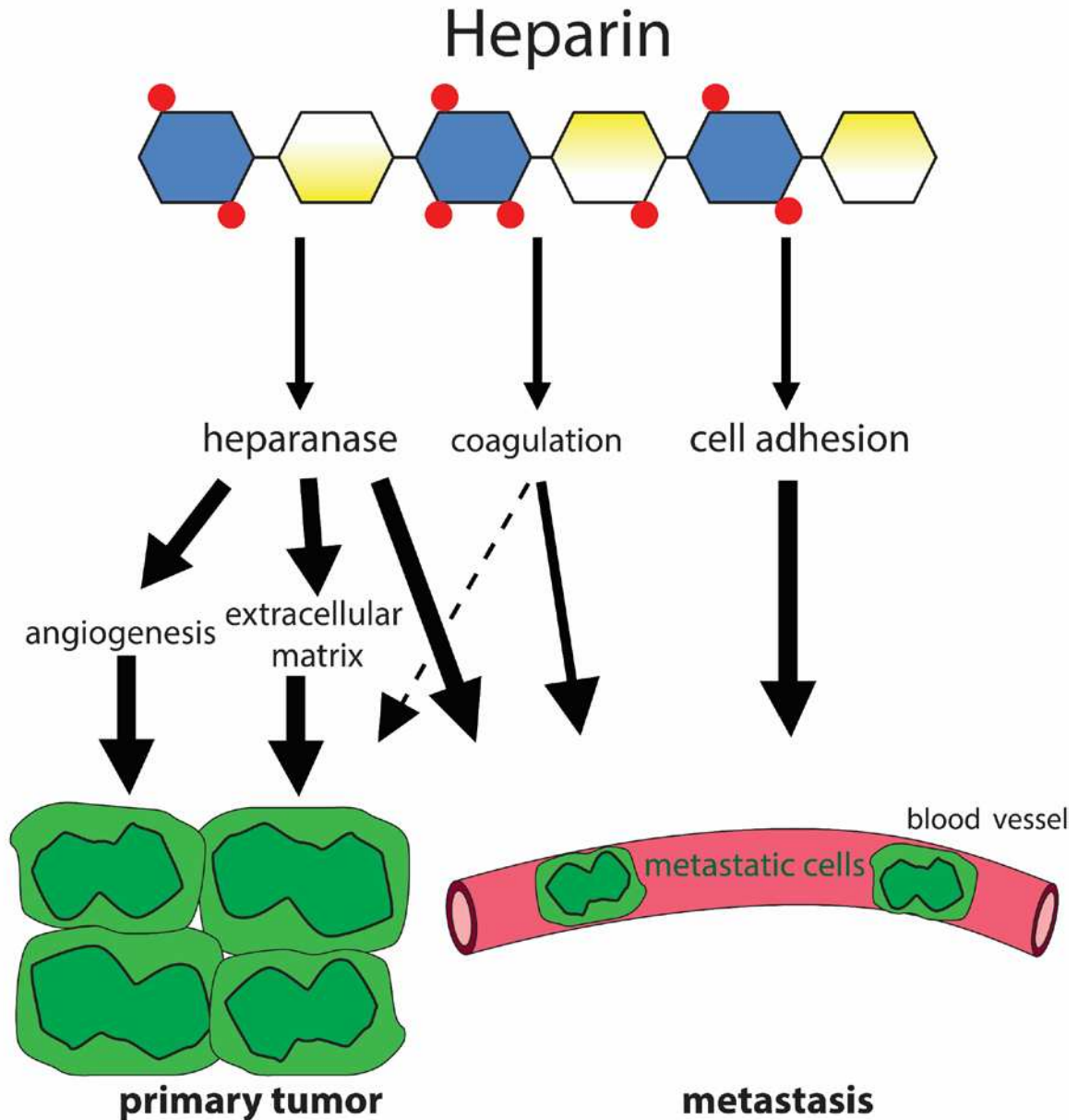
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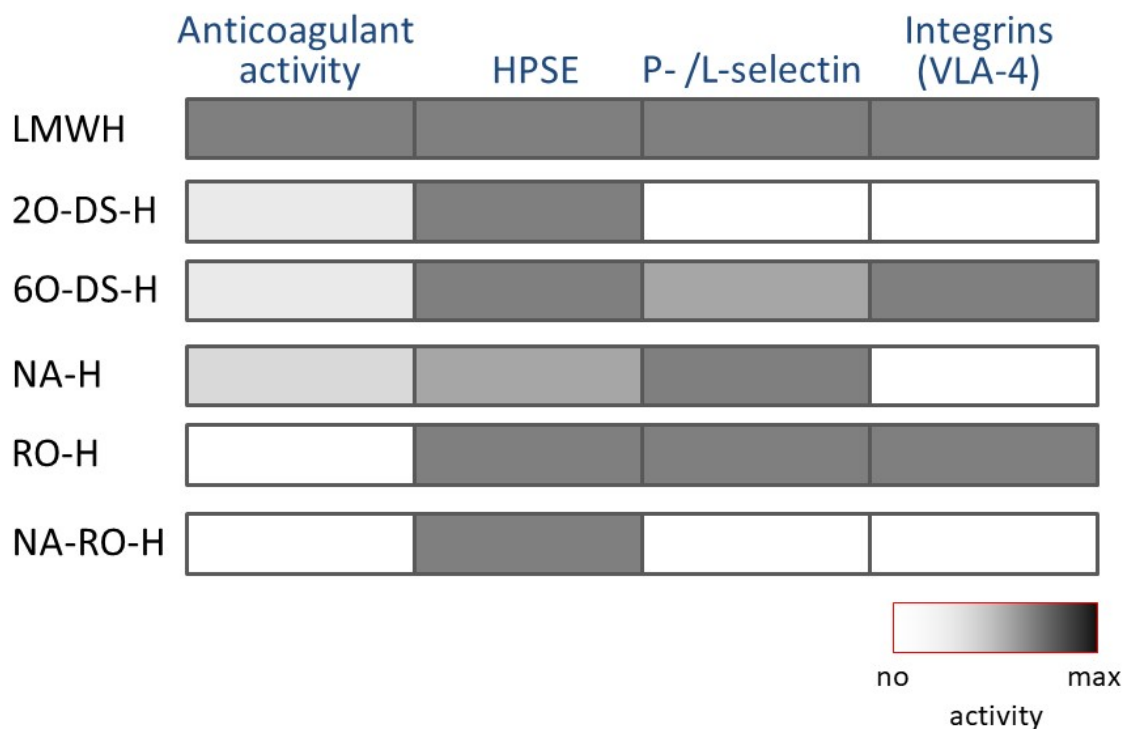
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## Figure Legends



**Figure 1** *Heparin contains diverse biological activities that affects cancer progression.* The main three biological activities affections heparanase (HPSE), coagulation and cell adhesion as discussed in this chapter. In addition, heparanase positively influences primary tumor growth through promotion of angiogenesis, and extracellular matrix remodeling associated also with a release of growth factors. Many of these activities have been confirmed *in vitro* and *in vivo*. A direct effect of heparin on metastasis has been confirmed through inhibition of heparanase and cell adhesion.

**Figure 2**



**Figure 2. Structural modifications of heparin and their impact on relevant targets during tumorigenesis and metastasis.** The anticoagulant activity of heparin is strongly attenuated or minimized by 2O- or 6O-desulfation; or by opening the iduronic acid ring (glycol-split heparin/RO-H), inhibitory capacity towards HPSE, P- and L-selectin or the integrin VLA-4 are largely preserved. Notably, N-acetylation of glycol-split heparin (NA-RO-H) appears to be a key to differentiate HPSE-inhibitory potential from the other targets.